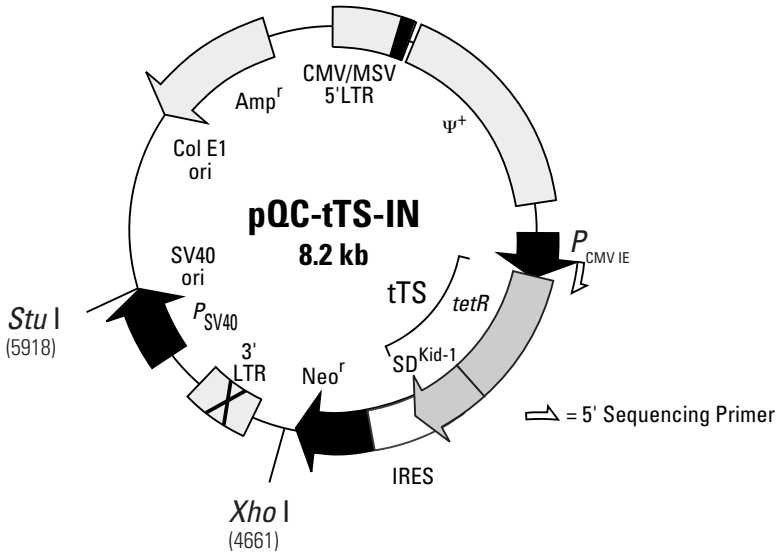
Cat. Nos. 630925
630926



Restriction Map of pQC-tTS-IN Vector. Unique restriction sites are in bold.

Description

The pQC-tTS-IN Retroviral Vector is a bicistronic expression vector designed to express the tetracyline-controlled transcriptional suppressor (tTS) along with the neomycin selection marker (1). Upon transfection into a packaging cell line, this vector can transiently express, or integrate and stably express, a viral genomic transcript containing the CMV immediate early promoter, tTS, IRES and the neomycin resistance gene (Neo^r). tTS and the neomycin resistance gene are cotranslated, via the internal ribosome entry site (IRES), from a bicistronic message in mammalian cells (2, 3). The tTS is a fusion of the Tet suppressor protein (TetR) and the KRAB-AB silencing domain of the Kid-1 protein (SD^{Kid-1}), a powerful transcriptional suppressor (4, 5). In the *absence* of Dox, tTS binds to the *tetO* sequences in the modified Tet-responsive promoter *(*P_{TREmod}*)* of a Tet response plasmid (pSIREN-RetroQ-TetH or pSIREN-RetroQ-TetP) and blocks expression of the shRNA. As Dox is added to the culture medium, the tTS dissociates from the P_{TREmod}, relieving transcriptional suppression.

This vector incorporates unique features including: optimization to remove promoter interference and self-inactivation. The hybrid 5' LTR consists of the cytomegalovirus (CMV) type I enhancer and the mouse sarcoma virus (MSV) promoter. This construct drives high levels of transcription in HEK 293-based packaging cell lines due, in part, to the presence of adenoviral E1A (6–9) in these cells. The self-inactivating feature of the vector is provided by a deletion in the 3' LTR enhancer region (U3). During reverse transcription of the retroviral RNA, the inactivated 3' LTR is copied and replaces the 5' LTR, resulting in inactivation of the 5' LTR CMV enhancer sequences. This mechanism may reduce the phenomenon known as promoter interference (10, 11) and allow more efficient expression.

Also included in the viral genomic transcript are the necessary viral RNA processing elements including the LTRs, packaging signal (Psi$^+$), and tRNA primer binding site. pQC-tTS-IN also contains a bacterial origin of replication and *E. coli* Amp^r gene for propagation and selection in bacteria.

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Use

The pQC-tTS-IN Vector is used to develop stable Tet tTS cell lines. pQC-tTS-IN is designed to deliver and express the regulatory protein tTS along with the neomycin resistance marker from a bicistronic message. After a pSIREN-RetroQ-Tet vector—containing an inserted shRNA under the control of a $P_{TREmod/U6}$—is transfected into a Tet tTS cell line, the tTS binds to the P_{TREmod}, suppressing transcription in the absence of tetracycline (Tc) or its derivative doxycycline (Dox). As Tc or Dox is added to the culture medium, the tTS dissociates from the P_{TREmod} and transcription of the shRNA is turned on in a highly dose-dependent manner. More information on pSIREN-RetroQ-Tet vectors and protocols describing the construction of Tet tTS cell lines can be found in the Knockout Inducible RNAi Systems User Manual (PT3810-1). The bicistronic transcript makes it unnecessary to screen the transformants since the neomycin resistance is expressed in concert with the DNA inserted into the multiple cloning site.

The design is optimized to produce high titers via the $P_{CMV\ IE}$ in the packaging cell line. Once transfected into the packaging cell line (such as the Retro-X™ Universal Packaging System [Cat. No. 631512]), RNA from the vector is packaged into infectious, replication-incompetent retroviral particles since pQC-tTS-IN lacks structural genes (gag, pol, and env) necessary for particle formation and replication; however, these genes are stably integrated as part of the packaging cell genome. Once a high titer clone is selected, these retroviral particles can infect target cells and transmit the gene of interest but cannot replicate within these cells due to the absence of viral structural genes. The separate introductioin and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

Location of Features

- 5' LTR (CMV/MSV): 1–728
  - Cytomegalovirus (CMV)/ mouse sarcoma virus (MSV) hybrid promoter:1–511
  - R region: 584–654
  - U5 region: 655–728
- Ψ^+ (extended packaging signal): 758–1567
- Cytomegalovirus (CMV) immediate early promoter ($P_{CMV\ IE}$): 1601–2132
- Tetracyline-controlled transcriptional suppressor (tTS): 2259–3107
- Internal ribosome entry site (IRES): 3115–3688
- Neomycin resistance gene (Neo^r): 3702–4496
- 3' MoMuLV LTR (deletion in U3): 4913–5338
  - Poly A signal: 5241–5246
  - cleavage site: 5261–5262
- SV40 promoter: 5618–5885
- SV40 ori (Site of replication initiation: 5839–5904
- Col E1 ori (Site of replication initiation): 6225
- Ampicillin resistance gene (β-lactamase): 7845–6985
  - Start codon (ATG): 7845–7843 stop codon (TAA): 6987–6985

Sequencing Primer Location

- pQC Seq/PCR Primer:
  - 5' Primer (2141–2164): 5'-ACGCCATCCACGCTGTTTTGACCT-3'

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to G418 (400 µg/ml).

Propagation in *E. coli*

- Suitable host strains: DH5α, DH10B, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: low

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Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with sequences obtained by Clontech Laboratories, Inc. This vector has been completely sequenced.

The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.